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The Structure and Growth of the Statocyst in the Australian Crayfish *Cherax destructor*

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Abstract. The morphology of the statocyst of the Australian crayfish *Cherax destructor* was examined using scanning electron microscopy. It resembles in general structure, size, and position the statocysts of crayfish described previously, and the size and distribution of the fields of setae on the floor of the capsule are similar but not the same. Over the size range examined, the relationship between the carapace length, the length of the basal antennular segment, the diameter of the statocyst capsule, and the total number of setae are all linear. The number and position of setae on the floor of the statocyst capsule were mapped for animals in two size classes (small, *ca.* 20 mm; large, *ca.* 50 mm) to test for changes in their arrangement during growth. The change in the ratio of setal number to statocyst size between the two size classes was about three times greater for the anterior setal field than for the other fields. We propose that differential development of the setal fields may be related to changes in the force-monitoring requirements of the animals as they increase in size, but this remains to be experimentally tested.

Introduction

Many decapod crustaceans have paired equilibrium organs called statocysts in the basal segment of each antennule. Statocysts monitor spatial orientation and movement (Cohen, 1955; Schöne and Neil, 1977; Sekiguchi and Terazawa, 1997). Each statocyst is a sac-like epidermal invagination of cuticle with a number of mechanosensory setae inside, mainly on the ventral floor. These are typically associated with a dense mass of sand, the statolith. The setae can be adjacent to the statolith and free to move, adjacent and touching, or cemented to the sand grains of the statolith. When the statolith deflects a seta it stimulates the neurons innervating it, and setae can differ in their physiological responses to stimulation (Cohen, 1955, 1960; Breithaupt and Tautz, 1988; Cate and Roye, 1997). The position and movement of the animal determine the pattern of setal stimulation, which in turn determines the form of compensatory movements made by the appendages and body (Sandeman and Okajima, 1972; Schöne and Neil, 1977; Patton and Grove, 1992b).

The morphology and spatial arrangement of setae within the statocyst vary between species (Cohen, 1955; Kovalev and Kharkeevich, 1993; Sekiguchi and Terazawa, 1997), and it has been suggested that groups of features may be associated with higher taxonomic groupings (Sekiguchi and Terazawa, 1997). In the statocyst of the crayfish *Orconectes limosus*, Hertwig *et al.* (1991) identified four separate fields of innervated setae: a lateral group of two semicircles, an approximately fusiform medial group with its axis roughly parallel to the long axis of the statocyst, and a single row of proximal setae. These setae appeared to be morphologically identical internally, but they differed in length and diameter in different parts of the field. Whether they differ in their physiological responses has not been tested.

Although both structure and function of crustacean statocysts are well understood, their growth has not been described as it has for other cuticular sensors on the crayfish and lobster tailfan (Letourneau, 1976; Schmitz, 1992; Stuart and Macmillan, 1997) and other appendages (Sandeman and Sandeman, 1996; Macmillan *et al.*, 1998; Steullet *et al.*, 2000). Growth in crustaceans occurs by periodic shedding of the cuticle, a process known as ecdysis, or molting, the body increasing in size with each molt. As the body grows, the sensory representation from the integument may need to change to maintain appropriate sensory input and function. As new sensory structures can only be added to the cuticle when the animal molts, a comparison of sensory structures

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in sequential molt stages reveals the order in which elements develop. Because of their accessibility, setae on the telson have been the subject of a number of developmental studies based on this principle. Letourneau (1976) found, for example, that the order of addition of sensory setae to the telson of *Procambarus clarkii* is a function of the growth of the animal. Schmitz (1992) described four functionally distinct setal types that are added at different rates. "Short smooth hairs" and "guard hairs" increase rapidly in number throughout development, whereas the number of two types of "feathered hydrodynamic hairs" remains relatively constant.

We describe here the basic structure of the statocyst in the Australian crayfish *C. destructor*, and the relationship between body size, basal antennal segment size, and statocyst capsule size over the size range of animals examined. We report the first data on the pattern of addition of setae within the capsule as the animal grows by comparing the statocysts from small and large individuals.

Materials and Methods

Individuals of *Cherax destructor* were obtained from a commercial hatchery at Bendigo, Victoria, Australia. They were kept in 50 × 20 × 120 cm aquaria under constant temperature with a normal 12-hour light/dark cycle, and were fed dried pellet food weekly.

Specimens with carapace lengths from 20 to 50 mm were examined. The animals were anesthetized by chilling in crushed ice for 30 min and were then decapitated. Statocysts were dissected from the dorsal surface of the basal segment of the antennules, and any extraneous tissue or adhesions were removed from around the cuticle of the statocyst with a fine paintbrush. The preparations were dehydrated in a series of ethanol solutions before being transferred to 100% ethanol for 12 h. After an additional 24 h in a desiccator, conducting graphite paint was used to glue the preparations to a scanning electron microscope stub. They were sputter coated with gold, and examined with a Phillips 505 scanning electron microscope. The images were processed using Adobe Photoshop Version 4.0. Measurements of carapace, basal segment of the antennule, and statocyst diameter were recorded for body index relationships, and comparisons were made using SYSTAT 6.0 for Windows.

Results

Location and general structure of the statocyst

The statocysts of *Cherax destructor* are in the dorsal region of the basal segment within the antennules (= first antennae; Fig. 1A, B). The statocyst is a cup-like invagination of the cuticle forming a cavity with a triangular, anteriorly facing opening on the dorsal surface. The opening is covered with a dense mat of setae that prevents entry of large foreign particles, effectively forming a closed capsule (Fig. 1B). The cavity itself is oval and slightly pointed posteriorly (Figs. 1C, 2A). The ventral floor of the cavity has an oval depression (Fig. 2A, B), and setae project dorsally through the cuticle adjacent to this. A statolith composed of fused sand grains sits in the depression (Fig. 2C).

Relationships between size of animal and size of antennule and cavity

The length of the basal segment of the antennule correlates closely with the carapace length ($n = 39$; $R^2 = 0.9711$; $P < 0.001$; Fig. 3A), so we were able to collect data on both body size and statocyst parameters from scanning micrographs of the local area. The length of the statocyst capsule increases linearly as a function of the size of the basal segment of the antennule ($n = 26$, $R^2 = 0.9546$; $P < 0.001$; Fig. 3A) and hence of the size of the animal.

Arrangement of setae and changes in distribution during growth

All of the setae on the base of the statocyst capsule of *C. destructor*, except those in the anterior part of the anterior setal field, are bound to the statolith (Fig. 2C). All setae that could be seen in scanning micrographs, because they were not obscured by the statolith, appeared to have the same external morphology (Fig. 2E), even though they varied in size. Because of the close association between the setae and the statolith, the process of removing it to examine the base of the capsule usually removed not only the setae but all associated tissues, including the tissues passing through the holes in the floor of the capsule. Remnants of these remained in a number of our preparations, however; these demonstrated that at least some of the setae are innervated through the holes in the base of the capsule (Fig. 2F). The presumption is that the holes represent innervation channels, as they do in other species (Hertwig *et al.*, 1991). The holes indicate the precise position of each seta on the floor of the capsule (Fig. 2A, B). Their disposition around the depression that normally holds the statolith resembles that in *Orconectes limosus* (Hertwig *et al.*, 1991), and direct correspondence with three of the four setal fields they described and named is apparent. A curved field made up of an inner double row and an outer single row forms a semicircle around the medial and posterior rim of the central depression. On the lateral side, this merges into the narrow end of a large triangle of setae occupying the area lateral to the rim of the depression. Opposite this large field, on the medial side of the depression, is a smaller triangular field. In an adult animal of around 50-mm carapace length, these fields are composed of about 68, 135, and 36 setae, respectively (Fig. 2D, Fig. 4) The total number of setae increases

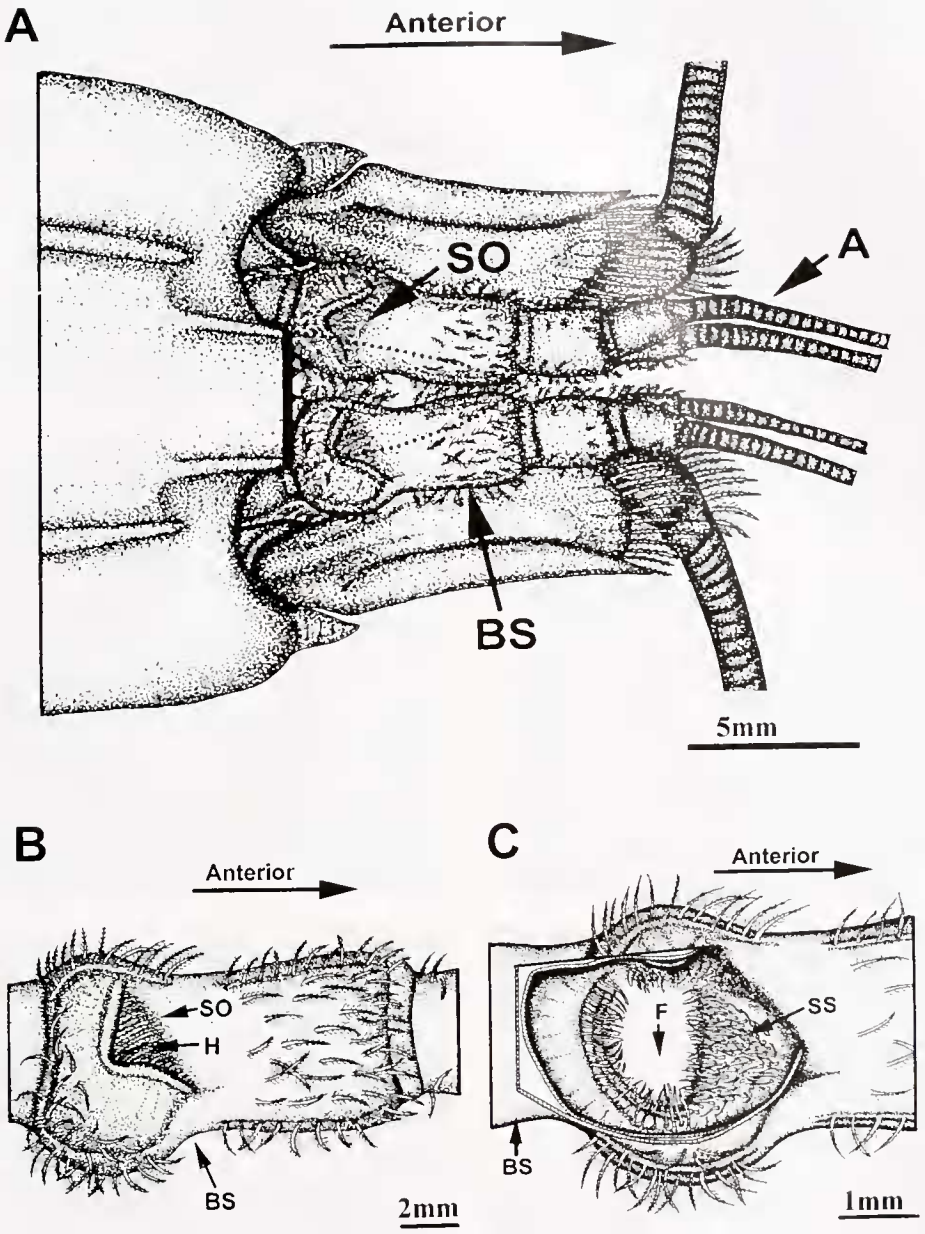

Figure 1. Morphology of antennular region and statocyst of the crayfish *Cherax destructor*. (A) Dorsal view of the basal segment (BS) of the antennule, and the location of the statocyst opening (SO). The rostrum and eyes have been removed. The position occupied by the rostrum is indicated by dotted lines. (B) Higher magnification of the basal segment (BS) of the antennule showing the dense screen of setae (H) that covers the statocyst opening (SO). (C) The statocyst capsule viewed through a window cut in the dorsal cuticle of the basal segment (BS) of the antennule to reveal the setae (SS) projecting upwards from the ventral floor (F) of the capsule. The statolith, with which all but the anterior setae make contact, has been removed.

linearly with the size of the animal ($n = 24$; $R^2 = 0.8663$; $P < 0.005$; Fig. 3B).

To examine the way in which this increase occurs, we counted the number of setae in a group of animals with a basal antennule length of 1.97 mm (SD = 0.19) ("small") and compared the result with a sample of animals with a basal antennule length of 5.75 mm (SD = 0.27) ("large"). The results of the survey are shown in Figure 4. A two-factor analysis of variance on the data testing for setal field type and size of animals showed that the large animals have significantly more setae in each field than the small animals ($F_{(1,50)} = 322.6$, $P < 0.01$), the number of setae in the three fields is significantly different ($F_{(2,50)} = 848.9$, $P < 0.01$), and the size of the difference varies between fields

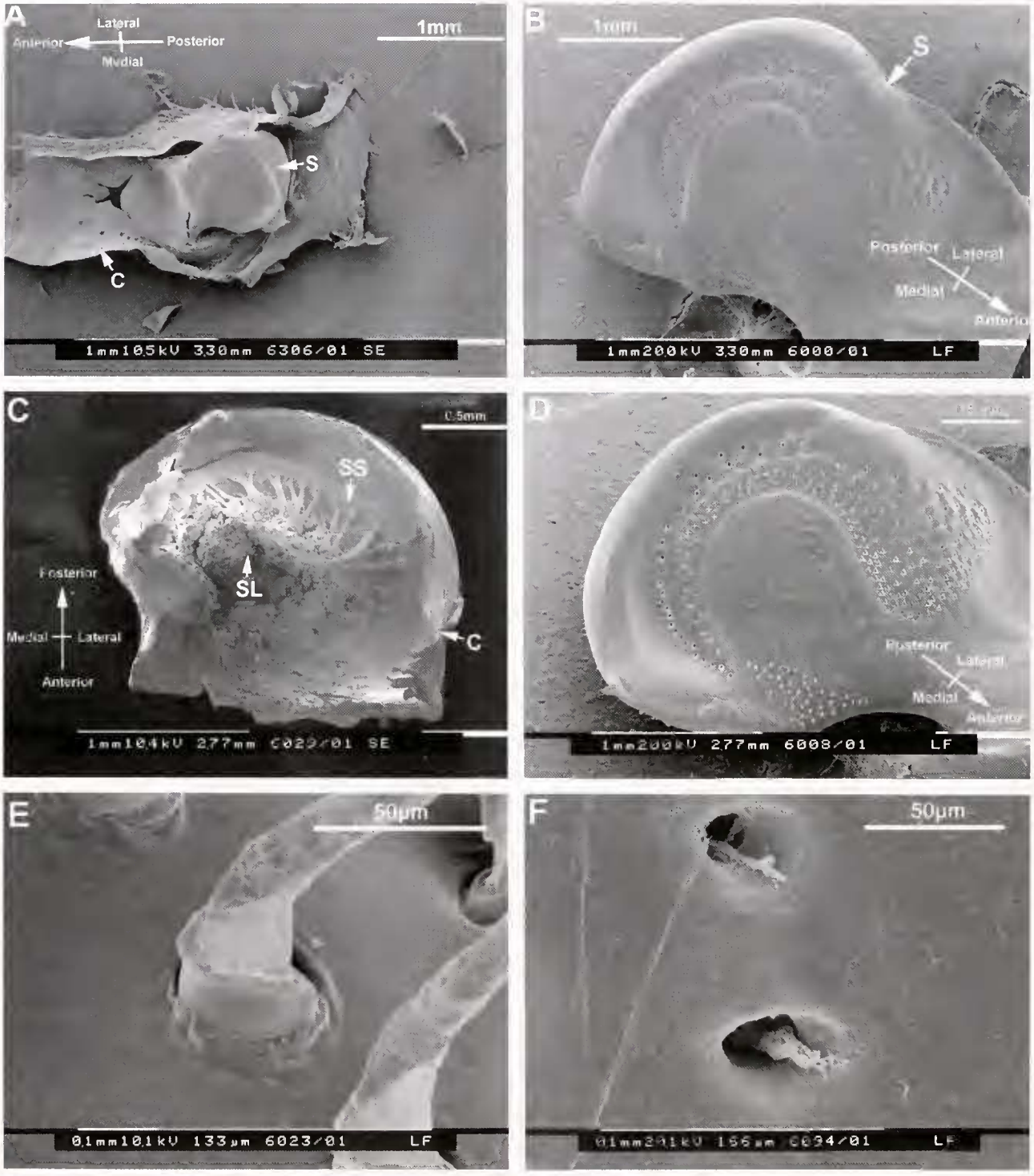

Figure 2. Scanning electron micrographs showing the statocyst of the crayfish *Cherax destructor*. (A) Dorsal view of the right antennule of an animal from the "small" group with part of the dorsal cuticle (C) cut away to reveal the floor of the capsule of the statocyst (S). The basal segment of this animal was 1.99 mm long. (B) Dorsal view of the floor of the statocyst (S) from the left antennule of an animal from the "large" group. The basal segment of this animal was 5.7 mm long. The magnification is the same in A and B so that the large increase in the number of setae in the anterior field is readily apparent. (C) Dorsal view of the statocyst capsule with part of the dorsal cuticle (C) removed to reveal the sensory setae (SS) in contact with the statolith (SL). Note that many of the setae in the anterior field do not contact the statolith. (D) Dorsal view of the ventral floor of the statocyst showing position of setae. The fields have been marked to correspond with the classification used previously in *Orconectes limosus*. △ Large anterior field (139 setae); curved field (69 setae); ○, outer row (29 setae); + inner rows (40 setae); ×, small field (46 setae). (E) High magnification view of base of a seta from the outer curved field viewed from the dorsal aspect. (F) High magnification view of the ventral surface of the same statocyst base as in E, showing holes beneath each seta and remnants of the mechanical and neural connections broken during the statocyst removal and preparation process.

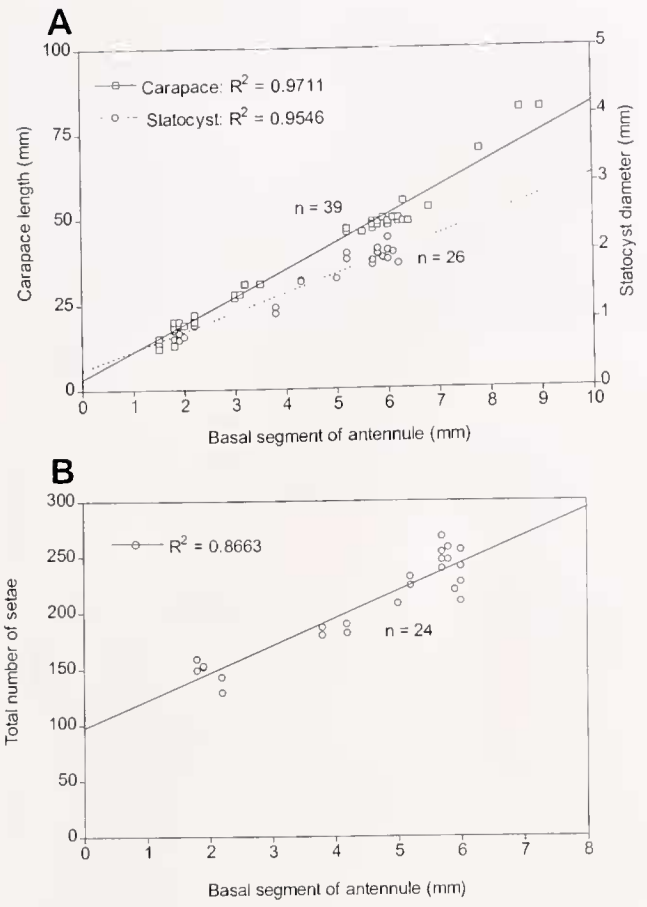

Figure 3. Statocyst size relationships. (A) Relationship between statocyst diameter, carapace length, and length of the basal segment of the antennule. The bold and dotted lines are the linear regression lines. Note the high correlation for both body measurement indices. (B) Relationship between length of basal segment of antennule and total number of setae within the statocysts. Note the high level of correlation between the base of the antennule (and hence body size) and the number of setae.

($F_{(2,50)} = 69.2$, $P < 0.01$). Tukey-Kramer pairwise comparisons between the three fields in both large and small animals showed that the number of setae is different in the three fields at the $P < 0.01$ significance level.

Discussion

The outcome of this work is straightforward. The result is a description of statocyst morphology in a previously undescribed crayfish species which permits some species comparisons to be made. In addition, this is the first report on changes in the size and setal arrangements of the statocyst with changing body size. The results therefore have implications for comparative and developmental questions.

In a mini-review, Sekiguchi and Terazawa (1997) compared information on statocysts across a range of crustacean species and found considerable morphological variation between taxonomic groupings but some evidence of consistency within them. The number of examples available, however, is probably not yet sufficient for a firm conclusion on this issue. The general morphology of the statocyst of *Cherax destructor* does appear, however, to be closely similar to that of other crayfish species examined (*Procambarus clarkii:* Takahata and Hisada, 1979; *Orconectes limosus:* Hertwig *et al.*, 1991). Because they used transmission electron microscopy as well as scanning electron microscopy, Hertwig *et al.* (1991) were able to show that all the setae on the floor of the statocyst capsule in *O. limosus* are morphologically identical. The external morphology of the setae in *C. destructor* suggests that they too may be of one type and probably even are closely similar to those in *O. limosus.* This does not, of course, mean that they are uniform in their physiological responses, because setae that appear closely similar may differ in their responses (Patton and Grove, 1992a). Irrespective of the way in which they transduce the detected forces into electrical signals, the positioning of different setae relative to the statolith must reflect the displacement forces that they can monitor (Cohen, 1955, 1960); thus the results suggest that further comparison of the arrangement of these elements across a range of species with differing lifestyles has the potential to reveal principles of statocyst structure and function.

Fortuitously, we were able to select specimens with a mean basal antennal segment length of 5.75 mm, which is close to the 5-mm length of *O. limosus* used by Hertwig *et al.* (1991), making comparisons between the fields in the two species less likely to be confounded by a size factor. They found four distinct groups of setae in *O. limosus.* Of these, three are clearly present in *C. destructor* and, in two cases, in comparable numbers: the curved field (*O. limosus: C. destructor* = 60:68), the large anterolateral field (135:<60), and the smaller medial field (30:36). The posterior line of 8 setae is not evident in *C. destructor,* but it is possible that they are part of the outer curved group but less

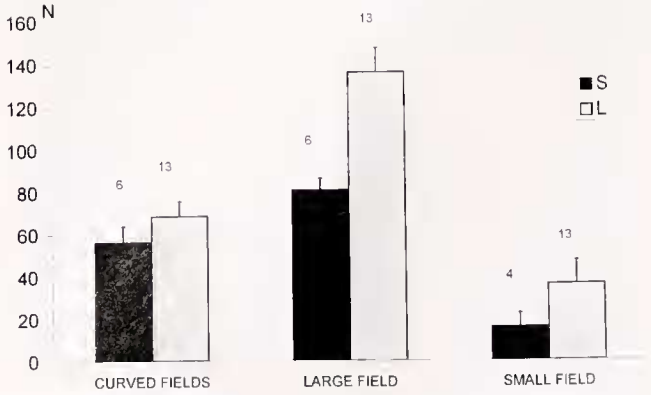

Figure 4. Comparison of the number of setae (*N*) in the different fields (curved, large, small) in a sample of small animals (S, mean basal antennal segment of 1.97 mm) and large animals (L, mean basal antennal segment of 5.75 mm).

distinctly separate than they are in *O. limosus*. If this were the case, the two species would both have about the same number of setae (*ca.* 68) in the posterolateral complex formed by these adjacent groups. In other decapod species studied, behavioral responses to stimulation of setae were found to correlate with the spatial location within the statocyst (Ozeki *et al.*, 1978; Kovalev and Kharkeevich, 1993). The curved and small fields occupy roughly the same position relative to the statolith in *O. limosus* and *C. destructor* and have approximately the same number of setae, all of which are attached to the statolith, an arrangement Cohen (1955) suggested as indicative of a prescribed output. It is therefore probable that they serve similar functions in terms of the requirements of the two species for positional information during behavior. In the lobster, setae in the lateral, posterior field respond to body roll, whereas setae anteriorly respond mostly to acceleration (Cohen, 1960). Although the argument rests on a body of cross-species data, it is likely that the body roll monitoring systems of *O. limosus* and *C. destructor* are similar. This then raises the interesting question of why the number of setae in the large anterior field differs so significantly between the two species.

In both *O. limosus* and *C. destructor*, the large anterior field differs from the other fields because many of the setae do not make contact with the statolith. Hertwig *et al.* (1991) argue that setae that are free of the statolith are most suited for detecting angular accelerations, an observation supported by behavioral observations in other species (Cohen, 1960; Patton and Grove, 1992a). The comparison of setal numbers in the different fields in the two size classes examined in our experiments showed that the ratio of change in the anterior free-field was about three times that seen in the small medial and the curved groups. Their rapid differential growth is therefore likely to correlate with the interaction between increasing body size and particular behavioral activities that involve a high degree of body mobility—activities such as three-dimensional movements in the water column or escape. The precise relationship between these behavioral considerations and the development of the statocyst remains to be determined.

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